Sesamol, a lipid lowering agent, ameliorates aluminium chloride induced behavioral and biochemical alterations in rats

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ABSTRACT

Background: Sesame oil from the seeds of *Sesamum indicum* Linn. (Pedaliaceae) has been used traditionally in Indian medical practice of Ayurveda in the treatment of central nervous system disorders and insomnia. A few published reports favor the anti-dementia effect of sesamol (SML), an active constituent of sesame oil. **Objective:** Thus, the present study was aimed to explore the anti-dementia effect and possible mechanism (s) of SML in aluminium chloride ($AlCl_3$)-induced cognitive dysfunction model in rodents with special emphasis on memory centers viz., hippocampus and frontal cortex. **Methods:** Male Wistar rats were exposed to $AlCl_3$ (175 mg/kg p.o.) for 60 days. SML (10 and 20 mg/kg) and rivastigmine (1 mg/kg) were administered orally 45 min before administration of $AlCl_3$ for 60 days. Spatial memory was assessed using Morris water maze test. After 60 days of treatment animals were sacrificed, hippocampus and frontal cortex were collected and analyzed for acetylcholinesterase (AChE) activity, tumor necrosis factor (TNF-α) level, antioxidant enzymes (Glutathione, catalase), lipid peroxidation, and nitrite level. The circulating triglycerides, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were also analyzed. **Results:** SML significantly prevented behavioral impairments in aluminium-exposed rats. Treatment with SML reversed the increased cholesterol, triglycerides and LDL while raised the HDL levels. SML significantly corrected the effect of $AlCl_3$ on AChE activity. Further, SML reversed the elevated nitric oxide, TNF-α and reduced antioxidant enzymes in hippocampus and frontal cortex. **Conclusion:** The present study suggests the neuro-protection by SML against cognitive dysfunction induced by environmental toxin ($AlCl_3$) in hippocampus and frontal cortex.

Key words: Aluminium, dementia, hypolipidemia, memory, sesamol, tumor necrosis factor-α

INTRODUCTION

Aluminium, a highly neurotoxic metal, is considered to be involved in the pathogenesis of neurodegenerative disorders like Alzheimer's disease (AD)[1-3] and Parkinson's disease.[4] Experimental animals, exposed to aluminium have developed AD-like conditions, characterized by elevated levels of amyloid beta (Aβ) protein and amyloid precursor protein (APP),[5,6] mitochondrial dysfunction, depletion of ATP,[7,8] induction of lipid peroxidation and lipid dystrophy,[9,10] accelerated production of phosphorylated tau,[11] impairment of cholinergic projections[12] and promotion of apoptotic neuronal death.[13,14] Thus, aluminium chloride ($AlCl_3$)-induced cognitive dysfunction model has been widely used for testing drugs against AD.[15-17]

Currently approved treatments for AD target neurotransmitter systems and only provide modest improvement in cognitive impairment. Thus, it is necessary to develop effective medications that go beyond acetylcholinesterase (AChE) inhibitors and N-methyl-D-aspartate antagonist. Several studies have demonstrated that hypercholesterolemia could cause dementia and Aβ_{42} deposition in hippocampal region.[18] Hypercholesterolemia is an outcome of sedentary life-style resulting in obesity and lipid dystrophy. Traditionally, in Indian medical practice of Ayurveda, sesame oil from the seeds of *Sesamum indicum* Linn. (Pedaliaceae) has been used to correct central nervous system disorders and insomnia.[19]

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Sesamol (SML), an agent obtained from sesame oil, edible oil, is found to reduce cholesterol and triglyceride levels in acute and chronic models of hyperlipidemia.[20] It is also reported to have antioxidant, neuro-protective,[21] anti-inflammatory[22] and hepatoprotective[23] activities. Although, all these actions of SML are beneficial to overcome the condition of dementia, SML has not been investigated for its behavioral effects in chronic models of dementia. In view of this, the present study was designed to investigate the effect of SML, a lipid lowering agent in $AlCl_3$-mediated behavioral and biochemical changes in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 200–250 g (90 days old) procured from Central Animal Research Facility of Manipal University, Manipal were used. Animals were acclimatized to laboratory conditions for 7 days before the experiment and they were maintained under controlled conditions of temperature (23°C ± 2°C), humidity (50% ±5%). The animals were kept under standard conditions of 12 h light/dark cycle in sanitized polypropylene cages containing sterile paddy husk as bedding with free access to food and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal [IAEC/KMC/73/2012] and was carried out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

Drugs and treatment schedule

Aluminium chloride (Spectrochem Pvt Limited, India), SML (Sigma-Aldrich Co, St. Louis, MO, USA) and rivastigmine (RIV) (Dr. Reddy's Laboratories, Hyderabad, India) solutions were made freshly on each day for administration. $AlCl_3$ was dissolved in distilled water and administered orally once daily at a dose of 175 mg/kg from day 6 onwards (24 h after the completion of retention trial on day 5) for 60 days. This dosing regimen for inducing dementia using $AlCl_3$ was determined according to the previous reports and the high rate of induction and low mortality, which was evident in the pilot study conducted. SML and RIV, at various doses, were administered 45 min before administration of $AlCl_3$ orally after suspending them in 0.5% sodium carboxy methyl cellulose (CMC) in distilled water for 60 days from day 6. On the basis of escape latency time (ELT) on day 5, animals were divided into six groups (*n* = 8). The groups were as follows:

Group 1: Normal control - Received distilled water (5 ml/kg *p.o.*).
Group 2: Vehicle control - Receives 0.5% CMC (5 ml/kg *p.o.*).
Group 3: $AlCl_3$ (175 mg/kg *p.o.*).

Group 4: RIV (1 mg/kg *p.o.*) + $AlCl_3$ (175 mg/kg *p.o.*).
Group 5: SML (10 mg/kg *p.o.*) + $AlCl_3$ (175 mg/kg *p.o.*).
Group 6: SML (20 mg/kg *p.o.*) + $AlCl_3$ (175 mg/kg *p.o.*).

The doses of the standard drug RIV (1 mg/kg) and the test drug SML (10 mg/kg and 20 mg/kg) were chosen based on the previous literature reports.[24-27] Body weight of the animals was taken on a daily basis before the treatments.

Spatial memory assessment using Morris water maze

To investigate the spatial learning and memory abilities of the experimental rats, Morris water maze task was performed as described by Morris[28] with minor modifications.[29] It consisted of a circular tank of 150 cm diameter and 40 cm height. The pool was divided into North-East, South-East, South-West and North-West (NW) quadrants. In the NW quadrant a hidden escape platform (10 cm diameter), was placed 2 cm below the water surface.

All rats were trained to find the escape platform. Animals were given four trials per day for 4 consecutive days. Animals were kept on the platform for 30 s and then removed. The rats that could not reach the platform in 20 s on the 4th trial-day were excluded from the study. On the probe day (day 5), the hidden platform was removed, and probe trial was performed with a cut off time of 60 s. All the animals were exposed to one retention trial on day 25, 45 and 65 to evaluate the memory consolidation. Data were acquired through a video camera connected to a computerized tracking system (Any Maze, Ugo Basile, Italy) fixed above the centre of the pool.

Dissection and tissue preparation

On day 65, immediately after the retention trial, the animals were sacrificed by decapitation. Brains were rapidly removed, hippocampus and frontal cortex were dissected according to the method described by Glowinski and Iverson.[30] A 10% w/v homogenate of samples was prepared by homogenizing with ice-cooled 0.1 M phosphate buffer potential of Hydrogen (pH) 7.4 using an ultra Turrax T25 homogenizer at a speed of 9500 rpm thrice at an interval of few seconds. The homogenates were then centrifuged at 15,000 rpm at 4°C for 15 min. Supernatant was collected and used for biochemical estimations.

Estimation of acetylcholinesterase activity

In the supernatant, AChE activity was measured by Ellman method using acetylthiocholine iodide as a substrate.[31] To a reaction mixture containing phosphate buffer (2.8 ml, pH 8), acetylthiocholine iodide (0.05 ml) and 0.05 ml of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) (Ellman reagent), 0.1 ml of the supernatant was added. The change in absorbance was measured for 4 min at 60 s interval at

412 nm using ultraviolet–visible spectrophotometer and the change in absorbance per minute was calculated. The results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein.

BIOCHEMICAL EVALUATION

At the end of the experimental period, animals were mildly anaesthetized with diethyl ether and the blood samples were collected by retro-orbital sinus puncture into microcentrifuge tubes. The tubes were then centrifuged at 10,000 rpm for 10 min at 20°C. After centrifugation, the serum was separated at once, divided into aliquots and stored at −20°C until they were used for biochemical analysis.

Collected serum samples were analyzed colorimetrically for triglycerides (glycerophosphate-oxidase-peroxidase (POD) method), total cholesterol (cholesterol oxidase-POD method), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels by end point method as per the manufacturer's instructions with the help of diagnostic kits (Aspen laboratories, Mumbai) using Enzyme Linked Immuno Sorbent Assay (ELISA) plate reader.

Estimation of lipid peroxidation and nitrite level
Estimation of nitrite level
Nitrite level in hippocampus and frontal cortex homogenate was measured by Griess reaction.[32] The extent of lipid peroxidation in hippocampus and frontal cortex was quantitatively determined by the method described by Konings and Drijver.[33]

Estimation of antioxidant enzymes
The catalase activity was determined by the method of Aebi *et al*., 1984[34] and glutathione (GSH) activity based upon the reaction between DTNB and sulfhydryl groups of GSH.[35]

Tumor necrosis factor-α Estimation
Level of tumor necrosis factor (TNF-α) in the supernatant was estimated by rat TNF-α kit as per the experimental protocol given by Invitrogen Corporation, USA. It involves a solid phase sandwich ELISA. The level of TNF-α was expressed as pg/mg of protein.

Estimation of total protein
Total protein was estimated in all tissue samples using Pierce® BCA Protein Assay Kit as per the experimental protocol given by Thermo Scientific, USA. Bovine serum albumin was used as a standard.

Statistical analysis
All the data are expressed as mean ± standard error of the mean. Results were analyzed by one-way analysis of variance , followed by Tukey's *post-hoc* test using Graph Pad Prism version 5.0 software. $P < 0.05$ was considered as statistically significant.

RESULTS

Body weight
After 60 days, $AlCl_3$ exposure and other treatment groups did not show a significant effect on body weight [Table 1].

Spatial memory assessment using Morris water maze
Time to reach hidden platform (Escape latency)
Aluminium chloride intoxication resulted in cognitive impairment as evidenced by significant increase in ELT during probe trials. On day 25, RIV and SML (20 mg/kg) produced a significant decrease in ELT when compared to $AlCl_3$-treated group. On day, 45 and day 65, all the treatment groups significantly improved the cognitive performance (i.e. decreased ELT) of animals relative to $AlCl_3$-treated group [Figure 1a].

North-West latency
Following 60 days of $AlCl_3$ administration, dementia was observed in rats as shown by the significant ($P < 0.05$) increase in the latency to find the target quadrant (NW). In RIV and SML (10 mg/kg and 20 mg/kg) groups, the latency to find the target quadrant was shortened significantly [Figure 1b].

Percentage time spent in target quadrant (north-west)
During the probe trials on day 25, 45 and 65, aluminium treated animals were found to spent significantly less time in the target quadrant (NW) as compared to control group. All the treatment groups significantly increased the time spent in the target quadrant relative to aluminium treated group during probe trial. SML (20 mg/kg) was found to have an activity comparable to RIV [Figure 1c].

Total zone entries
Aluminium treated group showed a significant decrease in total zone transitions on subsequent days of testing as

Table 1: Effect of $AlCl_3$ and $AlCl_3$+ treatments (RIV, SML) on the body weight of animals before and after treatment

Treatment	Day 1	Day 65
Control	227.6±2.75	294.0±9.80
Vehicle control	224.8±3.12	295.7±6.56
$AlCl_3$	230.1±3.60	267.3±6.93
$AlCl_3$+RIV (1 mg/kg)	230.0±3.65	292.0±7.26
$AlCl_3$+SML (10 mg/kg)	222.3±2.98	283.3±5.58
$AlCl_3$+SML (20 mg/kg)	222.3±3.80	287.8±6.24

Data presented as mean±SEM (*n*=8). *P*<0.05 as compared to control group and *P*<0.05 as compared to AlCl₃ treated group. SEM: Standard error of mean; RIV: Rivastigmine; SML: Sesamol; AlCl₃: Aluminium chloride
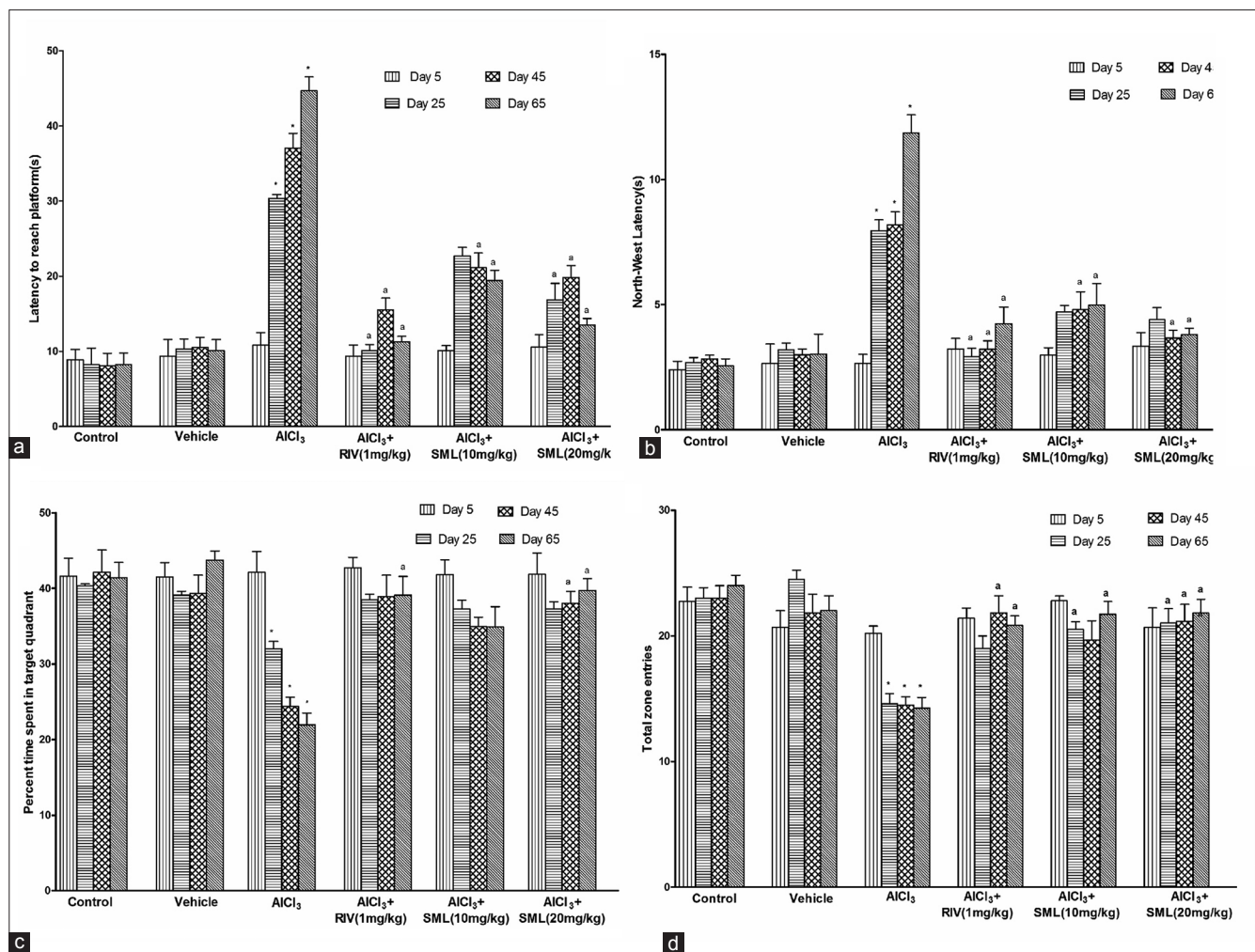
Figure 1: Effect of aluminium chloride (AlCl₃) and AlCl₃ + treatments (Rivastigmine, Sesamol) on (a) ELT time (latency to reach platform) (b) North-West (NW) latency (c) Percent time spent in target quadrant (NW). (d) Total zone entries and during retention trials before (day 5) and after (day 25, 45 and 65) treatment. Data presented as mean ± standard error of the mean ($n = 8$). *$P < 0.05$ as compared to control group and [a]$P < 0.05$ as compared to AlCl₃ treated group

compared to control group. RIV and SML significantly increased total zone transitions of animals as compared to AlCl₃ treated group [Figure 1d].

Acetylcholinesterase activity
Chronic AlCl₃ exposure significantly decreased AChE activity in the frontal cortex ($P < 0.05$) and hippocampus ($P < 0.05$) of rats as compared to normal control group [Figure 2a and b]. RIV also significantly ($P < 0.05$) decreased cholinesterase activity compared to control group in both hippocampus and frontal cortex. SML (10 mg/kg and 20 mg/kg) significantly reversed the effect of AlCl₃ on AChE activity.

Effect of treatments on lipid profile
Chronic administration of AlCl₃ for 60 days caused significant ($P < 0.05$) reduction in serum HDL levels [Figure 3a], increase in LDL levels [Figure 3b], total cholesterol [Figure 3c], triglycerides [Figure 3d] as compared to control group. Interestingly RIV significantly ($P < 0.05$) prevented the rise in LDL levels. Treatment with SML prevented the rise in total cholesterol, triglycerides and LDL levels and increased HDL levels as compared to AlCl₃ treated animals.

Estimation of lipid peroxidation and nitrite level
Estimation of malondialdehyde level
The malondialdehyde (MDA) levels in the hippocampus [Figure 4a] and frontal cortex [Figure 5a] of aluminium treated rats showed a threefold increase as compared to control group. The elevated MDA levels were significantly reversed by RIV and SML. SML at 10 mg/kg and 20 mg/kg dose level showed a better reduction of MDA levels than RIV in the hippocampus region.
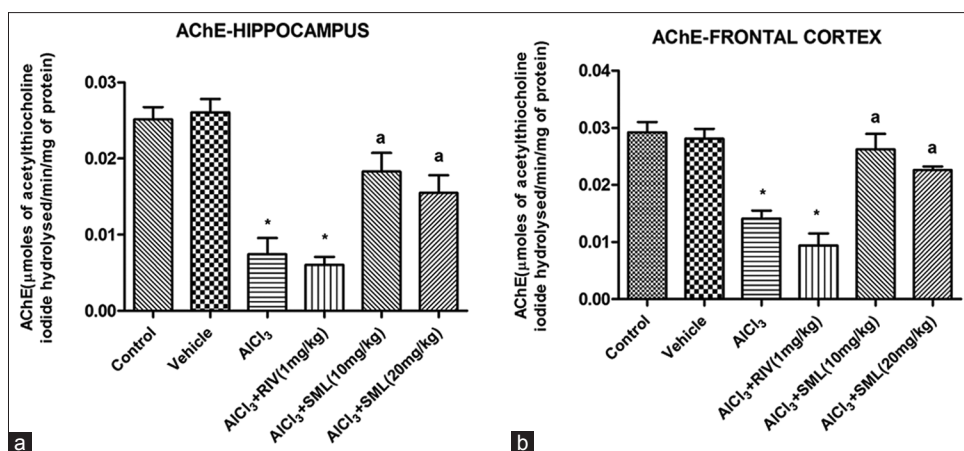
Figure 2: Effect of aluminium chloride (AlCl$_3$) and AlCl$_3$ + treatments (Rivastigmine, Sesamol) on acetylcholinesterase activity in (a) hippocampus (b) frontal cortex. Data presented as mean ± standard error of the mean (*n* = 8). *P < 0.05 as compared to control group and ᵃP < 0.05 as compared to AlCl$_3$ treated group
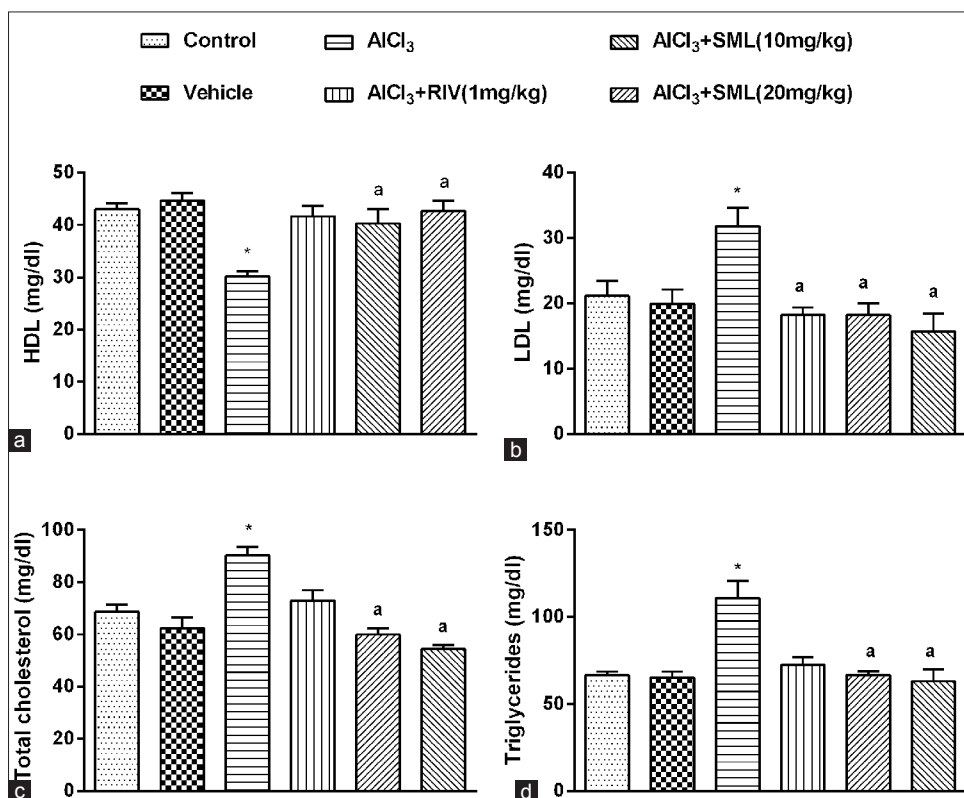

Figure 3: Effect of aluminium chloride (AlCl$_3$) and AlCl$_3$ + treatments (Rivastigmine, Sesamol) on (a) High-density lipoprotein (b) Low-density lipoprotein (c) Total cholesterol (d) Triglycerides. Data presented as mean ± standard error of the mean (*n* = 8). *P < 0.05 as compared to control group and ᵃP < 0.05 as compared to AlCl$_3$ treated group

Estimation of nitrite level

Chronic exposure of animals to AlCl$_3$ caused a significant elevation in nitrite levels in the hippocampus [Figure 4b] and frontal cortex [Figure 5b] as compared to control group of animals. RIV and SML (10 and 20 mg/kg) treatments significantly prevented the rise in levels of nitrite in both frontal cortex and hippocampus. In this case, SML (20 mg/kg) was found to reduce nitrite levels comparable to that seen in the control group.

Estimation of antioxidant enzymes
Catalase and glutathione activity

The hippocampus and frontal cortex of the AlCl$_3$-treated rats were observed to have significant (*P* < 0.05) reduction in catalase [Figures 4d and 5d] and reduced GSH activity as compared to control animals [Figures 4c and 5c]. RIV (1 mg/kg) and SML (10 mg/kg, 20 mg/kg) enhanced the catalase, and GSH levels significantly as compared to AlCl$_3$ treated group.
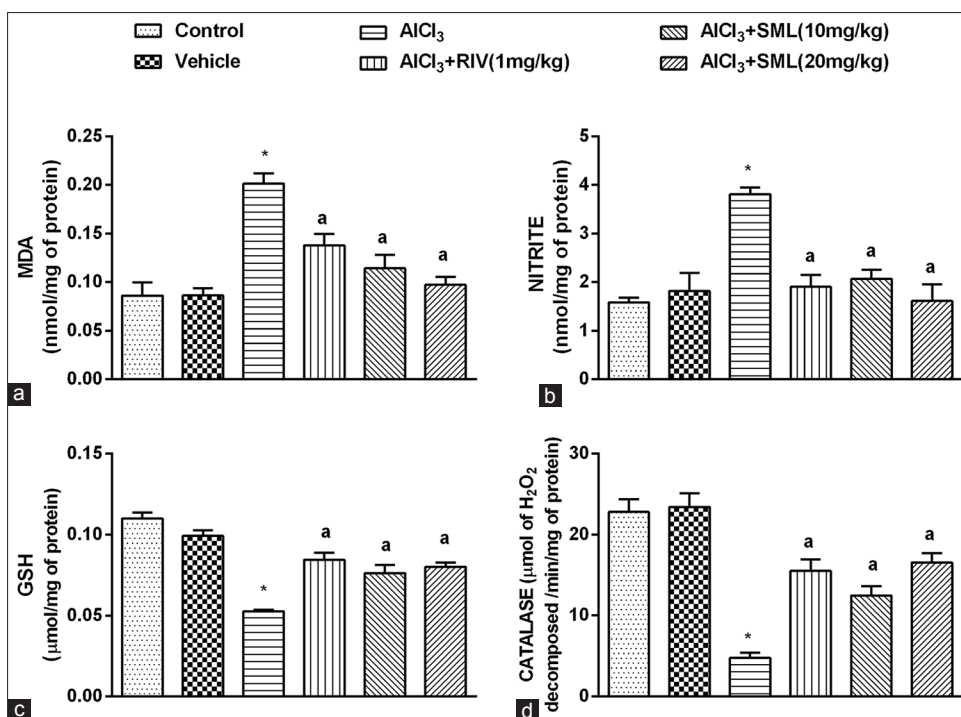
Figure 4: Effect of aluminium chloride ($AlCl_3$) and $AlCl_3$ + treatments (Rivastigmine, Sesamol) on hippocampus (a) malondialdehyde (b) nitrite level (c) glutathione level (d) catalase activity
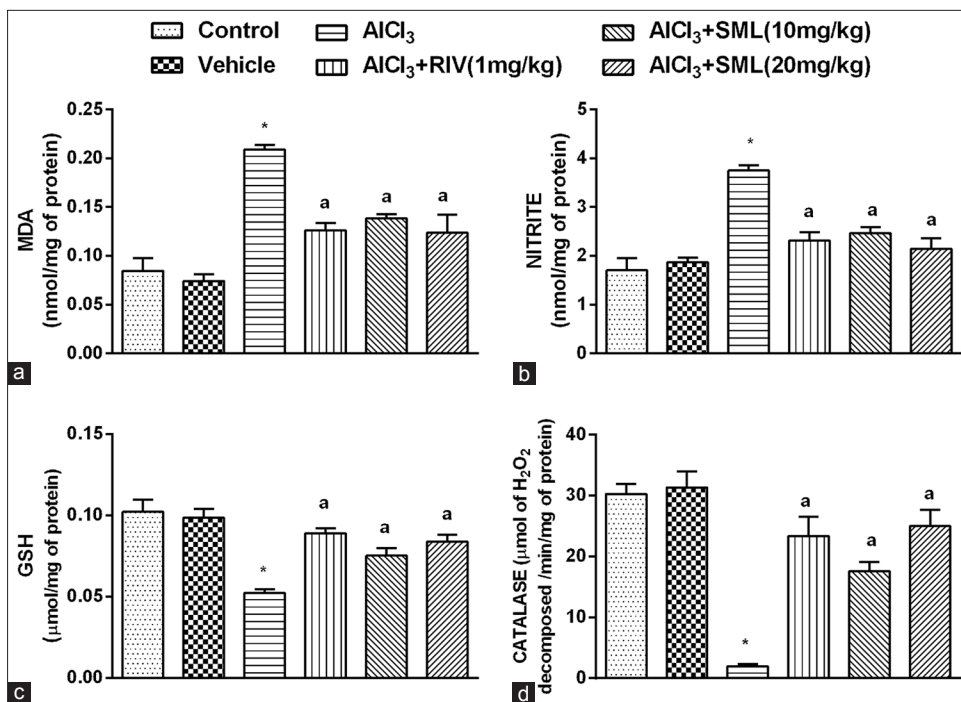

Figure 5: Effect of aluminium chloride ($AlCl_3$) and $AlCl_3$ + treatments (Rivastigmine, Sesamol) on frontal cortex (a) Malondialdehyde (b) Nitrite level (c) Glutathione level (d) Catalase activity. Data presented as mean ± standard error of the mean (n = 8). *P < 0.05 as compared to control group and [a]P < 0.05 as compared to $AlCl_3$ treated group

Estimation of tumor necrosis factor-α in hippocampus

Tumor necrosis factor-α levels were significantly (P < 0.05) increased (threefold) in the hippocampus of $AlCl_3$ treated animals as compared to vehicle group. Treatment with RIV (P < 0.05), SML (20 mg/kg) significantly (P < 0.05) inhibited this rise in TNF-α levels [Figure 6].
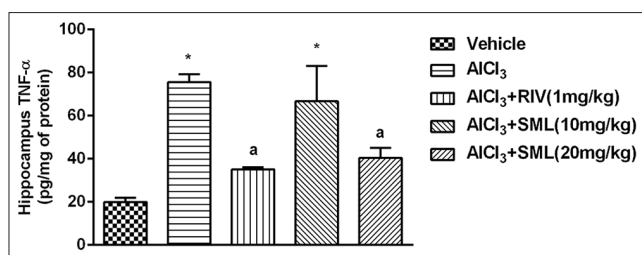
Figure 6: Effect of aluminium chloride (AlCl$_3$) and AlCl$_3$ + treatments (Rivastigmine, Sesamol) on tumor necrosis factor-α level in the hippocampus of rats. Data presented as mean ± standard error of the mean (*n* = 8). *$P < 0.05$ as compared to control group and ^{a}$P < 0.05$ as compared to AlCl$_3$ treated group

DISCUSSION

The study investigates the ameliorative effect of the lipid-lowering drug, SML, on AlCl$_3$-induced behavioral and biochemical changes in rodents. Aluminium was shown to accumulate in higher quantities in hippocampal and cortex regions, which are the sites of memory.[36] Spatial memory tasks are highly sensitive to hippocampus and frontal cortex[37] which is severely affected in neurodegenerative conditions such as AD.

Chronic aluminium exposure in animals was reported to cause cognitive decline.[38,39] Cognitive dysfunction is evident from decreased activity of experimental animals in Morris water maze,[24] radial arm maze[38] and passive avoidance task.[40] In the present study, the behavioral changes showed by aluminium exposed rats were inconsistent with previous reports. In Morris water maze test, aluminium exposure resulted in a significant decrease in spatial memory as indicated by increased ELT (time required to reach platform), NW latency (time required to reach target quadrant) and decreased percentage time in the NW zone and total zone entries during the probe trial. The treatment with SML and RIV reversed the memory deficit caused by AlCl$_3$. This suggests the beneficial effects of SML in correcting memory deficit associated with aluminium exposure.

Cholinergic system in the brain plays a major role in modulating learning and memory. Reduction in AChE activity and acetylcholine levels in hippocampus and cortex have been correlated with loss of cognitive function in AD patients.[41] Long-term potentiation in the hippocampal CA1 pyramidal neurons is modulated by AChE.[15] Moreover, it is essential for survival and growth of cells.[42] In experimental animals, aluminium has been shown to decrease AChE activity.[43] It shows a biphasic response on AChE activity, with an initial increase in the activity of the enzyme followed by a marked decrease. Formation of irreversible aluminium complex with high affinity toward the anionic site of enzyme and slow accumulation

of aluminium in the brain has been attributed for such biphasic response.[44,45] This explains the significant reduction in AChE activity in both hippocampus and cortex observed in our study after chronic AlCl$_3$ treatment. The toxic effect of aluminium may be attributed to reduced choline uptake,[46] erosion of cholinergic terminals in cortex and hippocampus,[47] and reduced choline acetyl transferase.[48] RIV, the standard AchE inhibitor showed further decrease in AChE activity thereby sustaining the action of the remaining acetylcholine from cholinergic neurons. SML was found to increase the AChE levels in aluminium-exposed rats; this may be attributed to the ability of SML to re-establish the acetylcholine release, thus protecting cholinergic neurons.

Apart from cholinergic deficit leading to memory impairment, effect of dyslipidemia on behavioral changes has been studied. In AD patients, elevation in the levels of total serum cholesterol and LDL-associated cholesterol has been implicated.[49] An increase in the membrane cholesterol enhances the lipid raft area, and the APP present in the rafts gets into contact with β-secretase very easily leading to increased Aβ production.[18] Aluminium through its dyslipidemic property could have contributed to a strong lipid membrane rafts in the brain neuronal membrane leading to AD like syndrome in rats. The dyslipidemia due to aluminium treatment (elevated levels of total cholesterol, LDL, triglycerides and decreased HDL levels) is largely attributed to the accumulation of aluminium in liver causing alteration in lipid metabolism.[50] In our previous study, SML was found to reduce both serum triacylglycerol and cholesterol levels.[19] In the present study, chronic treatment using SML was able to bring down the raised cholesterol, LDL, and triglycerides levels due to aluminium exposure. It also increased the HDL (good cholesterol) level comparable to control.

Oxidative stress and neuro-inflammation are involved in the pathology of neurodegenerative disorders.[51] Lipids are highly vulnerable to oxidative stress. The polyunsaturated fatty acids present in brain get attacked by the free radicals leading to the production of toxic aldehydes as 4-hydroxynonenal and acrolein which in turn, lead to conformational changes of proteins. Studies have suggested the possible involvement of Aβ induced lipid peroxidation in brain generating free radicals and reactive aldehydes resulting in neurodegeneration.[52,53] Nitric oxide (NO), a signaling molecule regulates many physiological functions in the body. It also acts as a free radical to induce nitrergic stress. The nitrergic stress in turn activates the mitochondrial pathway of apoptosis through up regulation of p53,[54] cytochrome c release[55] and through p38 mitogen-activated protein kinase pathway[56] leading to neuronal death. In our study,

we observed a significant increase in nitrite and MDA levels in aluminium treated group in accordance with the previous reports.[57] Further, aluminium treated rats showed a decrease in antioxidant system viz., catalase and GSH levels indicating considerable oxidative stress caused by the toxicant. Both RIV and SML treatment normalized the altered levels of nitrite, MDA levels and antioxidant enzyme like catalase, GSH. This may be due to the antioxidant effect of SML[58] and inhibition of NO synthase.[21,22] Ameliorative effect of SML on oxidative stress may be considered as one of the approaches to correct aluminium mediated neurotoxicity.

Accumulation of abnormal protein aggregates like $A\beta_{42}$ and free radicals (viz., nitrite, reactive oxygen species, reactive nitrogen species) may trigger cellular stress and neuroinflammation by activation of the brain's innate immune system involving microglia and astrocytes. Activation of these immune cells results in the release of inflammatory mediators such as TNF-α, interferon-α, Interleukin-6 resulting in neurodegeneration.[59,60] It has been observed that aluminium exposure has resulted in elevated TNF-α, a key cytokine which stimulates microglia to release glutamate causing excitotoxicity.[61,62] Similar to this we also observed a significant increase in TNF-α level in hippocampus following chronic aluminium exposure. This rise in TNF-α was counteracted by SML indicating its role in preventing neuroinflammation.

CONCLUSION

Sesamol treatment demonstrates a protective effect against AlCl$_3$-induced cognitive dysfunction in rats. The aluminium mediated biochemical changes were reversed, where SML enhanced AChE level in hippocampus and cortex regions through correcting hyperlipidemia, reducing oxidative stress, NO and TNF-α level. Further studies are awaited to establish the role of SML as a potential candidate to control neuronal disturbances.

ACKNOWLEDGMENT

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